THE RHYTHMIC ACTIVITY OF THE QUAHOG, VENUS MERCENARIA, AND ITS MODIFICATION BY LIGHT [1,2]

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The correlation of activities of animals in their natural environments with specific times of day, tide, and phases of the moon is an often-observed phenomenon. Also, it is a well-established fact that some of these activities, especially those correlated with the day-night cycle, persist even after the organism has been placed under conditions in which all factors known to stimulate the organism are kept constant (see reviews: Welsh, 1938; Park, 1940; Kleitman, 1949; and Webb, 1950). The cycles of activity which continue unaltered under constant conditions have been spoken of as endogenous or persistent rhythms as opposed to exogenous ones which continue only while the particular activating stimuli are being received by the organism. It has been suggested very strongly that the so-called endogenous rhythms are dependent on highly stable, inherent, physiological mechanisms or "internal clocks." However, the possibility that the animals that display persistent cycles of activity are actually reacting to some cyclically occurring physical forces of the external environment, in a manner not yet determined, has also been postulated by investigators.

Early in the present century, persisting or endogenous tidal cycles of activity were reported for certain marine animals. These papers have been reviewed by Brown, Fingerman, Sandeen and Webb (1953) who have demonstrated the existence of a persisting tidal rhythm superimposed upon the diurnal rhythm of color change in the fiddler crab, *Uca pugnax*. Moreover, these investigators have shown that in these crabs a semi-lunar or 14.8-day cycle of color change is the result of the precise relationship between the diurnal and tidal rhythms. More recent work illustrates that the rates of O_2-consumption of these crabs also reflect the presence of these rhythms (Brown, Bennett and Webb, 1954). Here it was found that the rate of O_2-consumption was generally greatest, other factors being equal, one to two hours before the times of low tides in the area from which the animals were collected. Again, it was clearly evident that the systematic moving of the tidal influence relative to the diurnal one produced characteristic patterns of O_2-consumption which recurred about every 15 days, or once every semi-lunar period. There was

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also a suggestion in the results of these studies that another cycle of O_2-consumption, a lunar or 29-day one, was present for this species.

The O_2-consumption rates of certain of the marine molluscs have also been correlated with environmental factors. *Patella, Mytilus,* and *Haliotis* were reported to have persisting rhythms of O_2-consumption under laboratory conditions (Gompel, 1937). Although the hourly data of these studies varied considerably, the highest rates of O_2-consumption were generally apparent about the times of high tides and lower rates were recorded at the times of low tides. More spectacular rhythms of O_2-consumption are described by Sandeen, Stephens and Brown (1954) for the marine gastropods, *Littorina littorea* and *Urosalpinx cinereus.*

Other molluscs exhibit tidal rhythms of motor activity when maintained under constant conditions. Bohn (1904) reported that *Littorina rudis* became active at 15-day intervals in the laboratory. In its natural habitat this snail is covered by water only during the semi-lunar high, high tides which normally occur about every 15 days. A persistent rhythm of locomotory activity related to the tides is displayed by *Nassa obsoleta* (Stephens, Sandeen and Webb, 1953). Also, the filtering rate of species of *Mytilus,* using colloidal graphite, varies rhythmically in phase with the tidal cycles in the area from which the mussels were collected (Rao, 1954).

Some of the characteristics of these persistent rhythms have been elucidated by various experimental procedures of which light and temperature modifications have been employed most often. The effects of changes in light on the diurnal rhythm of color change for fiddler crabs are discussed by Brown and Webb (1949), Webb (1950), Brown and Hines (1952), and Brown, Fingerman and Hines (1954). Brown and Stephens (1951) have reported modifications of this cycle which were induced by photoperiod. The only paper to date which describes the experimental modification of a tidal rhythm by changes in illumination is that of Brown, Fingerman, Sandeen and Webb (1953). These investigators found that when the diurnal color change rhythm for *Uca pugnax* is shifted by an appropriate light stimulus, the tidal rhythm is shifted correspondingly. Temperature studies have demonstrated that the frequencies of both the diurnal and tidal rhythms of color change for *Uca* are temperature-independent over fairly great ranges, at least 6° C. to 26° C. for the diurnal cycle (Brown and Webb, 1948), and at least 13° C. to 30° C. for the tidal cycle (Brown, Webb, Bennett and Sandeen, 1954). The tidal cycle of pumping for *Mytilus* is independent of temperature in the range 9° C. to 20° C. (Rao, 1954).

The remarkable stability of the so-called endogenous rhythms has supported the hypothesis that the possession of these cycles is of adaptive significance for these animals. Thus, they are able to anticipate the time of day or tide, or phases of the moon. Organisms whose feeding activity and/or breeding periods seem to be correlated with specific times in these cosmic cycles would appear to be likely subjects for the study of biological rhythms.

Korringa (1947) was able to show that the time of spawning of oysters and several other lamellibranchs exhibits a lunar periodicity. The results of studies on oysters (Korringa, 1952) and quahogs (Loosanoff, 1939) in their natural habitats, however, have been interpreted as indicating that these animals do not display any persisting activity correlated with the time of day or tide.

It was the purpose of the present study, therefore, to determine whether or not the common quahog, *Venus mercenaria,* an animal of the intertidal zone and shallow

water, exhibits activity cycles under constant laboratory conditions. The activity rhythms that were observed have been related to the day-night cycle, to the phases of the tidal cycles, and hence to lunar periodicity. Also, an attempt was made to modify the character, or phase relationships to time of day, of the cycles of activity by light changes.

Material and Methods

The activities of three groups of quahogs were studied in Evanston, Illinois, during the winter and spring of 1953. They were obtained from a local fish market, and had been collected originally in the Chesapeake Bay region of Virginia. A fourth group was studied during June, 1953; these were clams which had been collected in Little Harbor, Woods Hole, Massachusetts. In December, 1953, the activities of two more groups of quahogs were studied concurrently in an attempt to ascertain the effects of light changes on the persistent cycles of activity. These animals again were obtained from a local market in Evanston, and had been collected in Virginia.

All the clams used for the activity studies were adults. There were eight to ten animals in each group; additional clams, which were obtained at the same time as those whose activity was being recorded, were kept under the same conditions as those that were being studied. In the event that a clam died, one of the additional animals was substituted, and the activity records of the original animal were discarded. Over the course of the observations, the mortality rate was very low. The great majority of the quahogs were in good condition even after having been in the laboratory for as long as four weeks.

The quahogs were placed in finger bowls containing sea-water which was changed at irregular intervals so that any observed rhythms could not be attributed to a regular time of water change. During the course of the study of the first four groups, the illumination at the surface of the water, as measured with a Weston photometer, was constant at approximately 2 ft. c. The source of illumination was fluorescent lights. The temperature of the water varied irregularly between 20° C. and 22° C.

Steel wires were bent to fit snugly over one of the valves of each animal from umbo to ventral edge. To these wires were tied threads which, in turn, were connected to sensitive heart levers or to the levers of the automatic recording spring scales described by Brown (1954). These levers recorded the opening and closing movements of the clams continuously on slow kymographs for periods of two to three weeks.

The daily record of each clam was analyzed by determining the times of opening and closing and the duration of the period open or active. The results obtained from the eight to ten animals of a group were averaged to obtain values for the total amount of time open per day. The time open was plotted against the days.

The following procedure was followed in the study of the effects of changes of illumination on the rhythms. One group of animals, the control group, was maintained under constant illumination of 2 ft. c. The experimental group was kept in a photographic dark room. For five consecutive nights (8 p.m. to 8 a.m.) this group was exposed to fluorescent lights providing an intensity of 100 ft. c. During the day (8 a.m. to 8 p.m.) they were kept in darkness. From 8 p.m. of the last day of darkness until the end of the experimental period, they were maintained at

an illumination of 2 ft. c. During the three-week period, the temperatures of the two rooms in which the clams were kept were recorded continuously on Taylor thermographs, and were seen to fluctuate between 20° C. and 22° C. The records for these animals were analyzed as were those for the clams of the first four groups. However, the average values for three consecutive days were averaged and this average used as the value for the second of the three days, *e.g.,* the figures for December 4, 5, and 6 were averaged and the resulting value used as the time open for December 5. Such procedure was used to obtain the relationship illustrated in Figure 5, and was thought to render the comparisons between the experimental and control curves more significant.

The form of the average diurnal curve for each group was ascertained by averaging the times open or active for each of the 24 daily, one-hour periods of 15 consecutive days. These 24 values were converted further to the percentage of 60 minutes open or active. All phases of a possible tidal influence are represented in
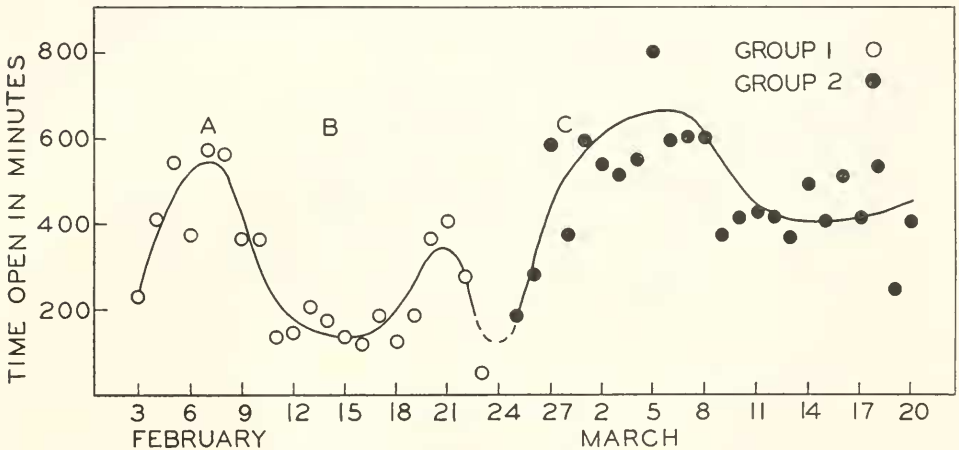

FIGURE 1. The long-term cycles of activity for group 1 (open circles) and group 2 (black circles) which were studied in Evanston, Ill. For explanation of A, B, and C, see text.

each of the values, and are thereby randomized since data for 15 days were used. In the cases in which the particular observational period was longer than 15 days, a 15-day period was arbitrarily selected, usually from the middle of the period.

Conversely, the diurnal rhythm was rendered random to reveal the character of the tidal rhythm in the following manner. The values for activity of the 24 daily, one-hour periods for each of 15 successive days were moved backwards at an average rate of 50 minutes a day, *i.e.,* on succeeding days later one-hour periods became aligned with earlier ones. By this means any possible tidal cycles were kept in phase with one another in the vertical columns of data. By continuing this process, repeating with the data of the first 14 days until a total of 29 days had been included, every phase of a diurnal influence was moved across the whole 24-hour period. Again, each of the 24 values has been expressed as the percentage of 60 minutes active or open. In Figure 4 the percentage of each hour open was plotted against time in hours in such a manner that the first minimal point fell between the sixth

and seventh hours. This procedure was obviously perfectly legitimate since the points on the abscissa merely indicated consecutive hourly periods, and do not represent actual times of any given day.

<div align="center">RESULTS</div>

1. The studies of animals maintained under constant conditions

A. The long-cycle rhythm

The average time open in minutes per day was determined for each group of quahogs. Long-cycle rhythms of activity were rendered clearly evident by plotting the time open (ordinate) against the date (abscissa). These cycles of activity for the four groups of animals are illustrated in Figures 1 and 2. It can be seen in the curve for groups 1 and 2 (Fig. 1) that major peaks of activity occur about 29 days apart and a minor peak occurs about half way between. The curve for group 3 (Fig.
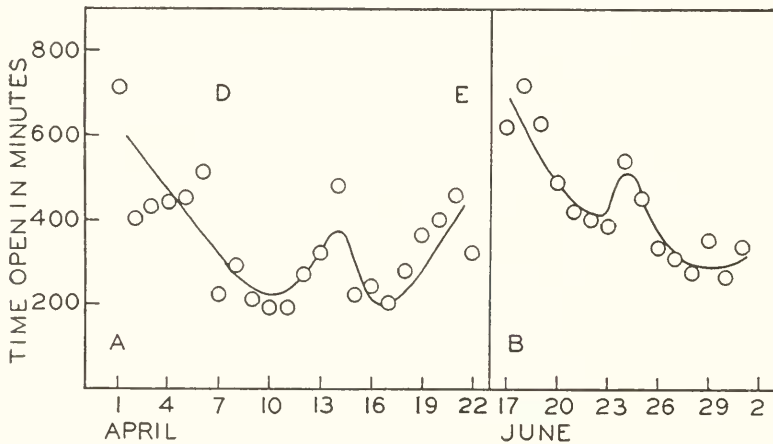

FIGURE 2. The long-term cycle of activity for group 3 (A) which was studied in Evanston, Ill., and that for group 4 (B) which was studied in Woods Hole, Mass. For explanation of D and E, see text.

2A) and for group 4 (Fig. 2B) show the minor peaks of a cycle with periods of lower activity on either side. It is also evident that the curve for group 3 can be superimposed on those for groups 1 and 2 from points A to C in Figure 1. Furthermore, the curve for group 4 simulates those for groups 1 and 2 from points B to C in Figure 1, and also that for group 3 from D to E in Figure 2A.

It was thought that the clams in each of the first three groups were probably in synchrony with one another since they were reported to have been collected in the same area of the Virginia coast. Examination of the activity records of the individual animals of a single group supports this assumption. Within a group, all the individuals have their maxima and minima occurring within a particular 48-hour period. In group 4, the variation is even less. With the exception of one animal, all the latter showed their highest observed activity on June 17 and a secondary peak of activity on June 24. All the clams of this group had their lowest observed activity

from about June 27 to June 29, correlated with the time of the semi-lunar low, low tides in Little Harbor.

B. The diurnal rhythms

The diurnal rhythms for the four groups of animals are shown in Figure 3. The average percentage of an hour open for each of the 24-hour periods was plotted against the time of day in Eastern Daylight Time, so that the cycles of the Evanston and Woods Hole groups could be compared directly.

Most evident in the curves of the diurnal rhythms are the facts that in all the groups, the maximal activity occurred in the afternoon, and that activity reached a
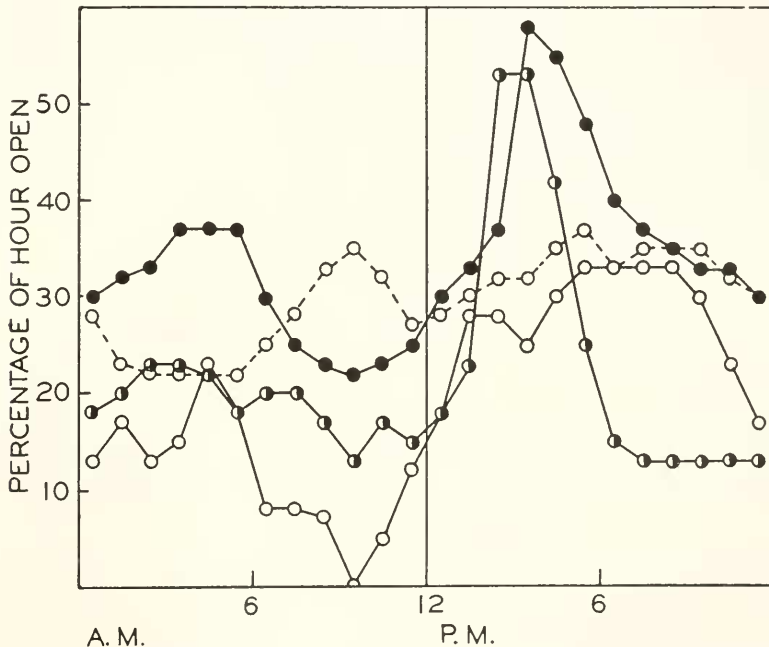
FIGURE 3. The diurnal rhythms of activity for the four groups of animals that were studied under constant conditions. Group 1 (open circles with solid lines), group 2 (black circles with solid lines), group 3 (black and open circles with solid lines), and group 4 (open circles with broken lines).

low point during the morning hours. The afternoon maximum was recorded between 5 and 9 p.m., 3 and 4 p.m., 2 and 4 p.m., and 5 and 6 p.m., for groups 1, 2, 3, and 4, respectively. In groups 1, 2, and 3, the morning minimum occurred between 9 and 10 a.m. whereas in group 4, the time of minimal activity extended from 2 to 6 a.m.

The diurnal rhythm of group 3 is striking in that a relatively long period of low activity is apparent between 7 p.m. and midnight whereas in groups 1, 2, and 4 the activity fell gradually from the afternoon highs during a comparable period of time. All groups of clams also had a minor peak of activity during the morning hours. In

the first three groups these peaks were between 4 and 5 A.M., 3 and 6 A.M., and 2 and 4 A.M., respectively. The morning maximum in group 4 did not occur until 8 A.M. although the morning low occurred earlier, *i.e.,* between 2 and 6 A.M., than in the other groups. The minor peak of the morning occurred about 12 hours before the diurnal maximum in all the groups, except group 4.

C. The tidal rhythms

The tidal rhythms of activity, analyzed as described earlier, are illustrated in Figure 4. Two periods of lower activity are evident in each of these curves. These two periods of each curve are about 12 hours apart, thereby approximately reflecting the normal frequencies of the tides of the Atlantic coast. Also to be noted is the fact that the low points of the curve for group 4 occur near the time of low tides in the area of collection. This was the only group for which correlations could be made between actual tidal events and phases of the activity cycles. Since in rendering the diurnal cycles random in order to ascertain the tidal cycles, the successive tidal cycles were brought into phase with the tidal cycle of June 17, 1953, tidal conditions on this day are the ones which should be correlated with the resulting tidal curve. When the points were moved so that the first minimum came to lie between hours 6 and 7, the indicated times of the low tides were moved correspondingly.
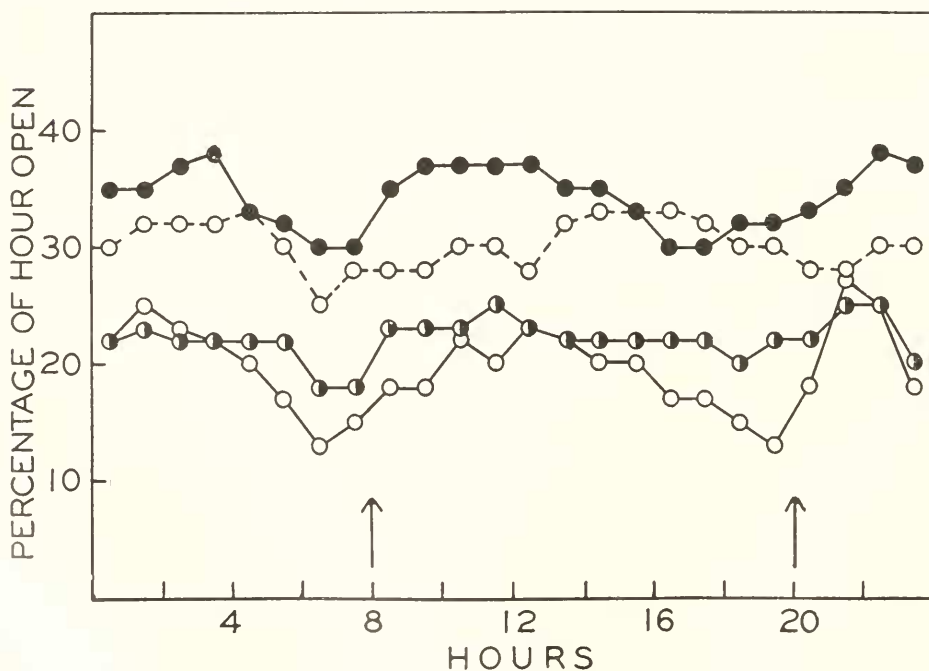

FIGURE 4. The tidal rhythms of activity for the four groups of animals that were studied under constant conditions. Group 1 (open circles with solid lines), group 2 (black circles with solid lines), group 3 (black and open circles with solid lines), and group 4 (open circles with broken lines). Note that the two periods of low activity for group 4 were near the times of low tide as indicated by the arrows.
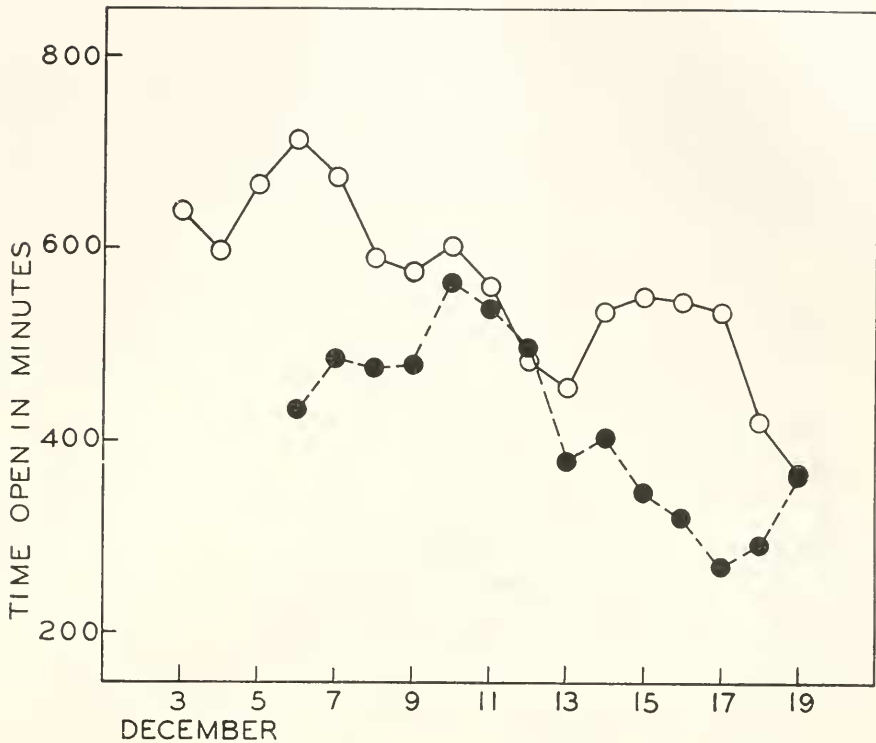
FIGURE 5. The long-term cycles of activity for the group of clams that was exposed to an illumination of 100 ft. c. by night and darkness by day for five consecutive days (broken lines) and for its control group that was maintained under constant laboratory conditions (solid lines).

The particular method used in determining the general character of the tidal cycle does not permit demonstrating precisely the expected 12.4-hour character of the tidal cycles. The curve for group 1 does show two minima occurring 13 hours apart while that for group 2 has low points 10 hours apart, group 3 11.5 hours apart, and group 4 14.5 hours apart. The average obtained, therefore, was 12.25 hours for the four series.

The amplitude of the four cycles also differs from one to another. That for group 1 has the greatest amplitude, an increase of 108% from lows to highs, while the other three increased only 27% to 32% from low points to high ones. Another interesting point evident in the curves of the tidal cycles is that in all of them one of the two periods of increased activity shows two peaks of relatively high activity separated by two to three hours of relatively low activity.

The shapes of the tidal curves for groups 1, 2, and 3 are similar to that illustrated for group 4 for which correlations with actual tidal phases could be made. Therefore, it would be reasonable to predict tidal events in the habitats of groups 1, 2, and 3, since the phases of the persistent tidal cycle probably always bear corresponding relationships to the times of tides in the native habitat.

II. The effects of reversed illumination on the activity cycles

The effects of reversed illumination, *i.e.*, 100 ft. c. from 8 P.M. to 8 A.M. and darkness from 8 A.M. to 8 P.M. for five days, on the long-cycle activity are presented in Figure 5. By comparing the curves for the experimental group and its control, it appears that the illumination changes effected an over-all lower activity for the treated group. The average time open per day for the 20-day observational period is 574 minutes for the controls and only 432 minutes, or about 25% less, for the experimental group.
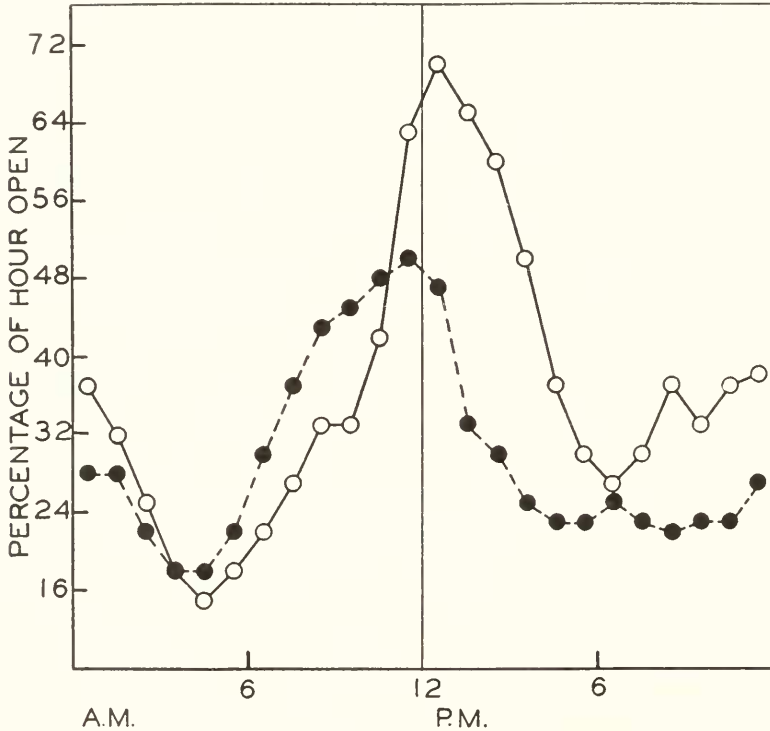

FIGURE 6. The diurnal cycles of activity for the group of clams that was exposed to reversed illumination (broken lines) and for its control (solid lines). The cycle for the experimental group appears to have been shifted one hour to the left or earlier when compared to that for the control group.

A change in the temporal relationships of the curve for the experimental group is also evident in Figure 5. This shift can be seen best by comparing the high point of December 6 on the control curve with a comparable high on December 10 on the experimental one. Low points also occurred four days apart, *i.e.*, on December 13 for the controls and on December 17 for the treated animals.

The general form of the long-term cycle of the control group, as well as that of the experimental group, was seen to be similar to the general form of the long-term cycles of activity that were found for the first four groups of quahogs (Figs. 1 and 2). It is probable that the portion of the cycle from December 13 through

December 19 of the control curve (Fig. 5) represents a minor peak which corresponds to that of groups 1 and 2 between February 18 and 24 (Fig. 1), to that of group 3 from April 11 through 17 (Fig. 2A), and that of group 4 from June 22 through 28 (Fig. 2B). It seems highly probable that the points on the control curve (Fig. 5) prior to December 13 represent the descent from a major peak. If this is true, a major peak would have occurred 15 days earlier or about December 1. A high value recorded for December 1 supports this view. However, this was the first day that the quahogs were in the laboratory and therefore the value was not incorporated in the figure.

The diurnal rhythms for the two December groups were strikingly similar to one another (Fig. 6). Again, the greater activity of the control animals is evident in this figure, and it is seen that the larger part of this higher activity is contributed by a greater diurnal maximum. The curve for the experimental group shows merely a slight shift in temporal characteristics when it is compared with the control
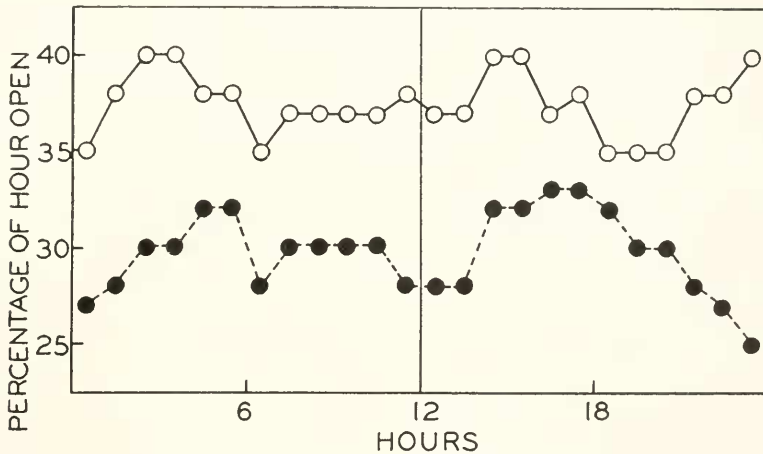

FIGURE 7. The tidal cycles of activity for the group of animals exposed to reversed illumination (broken line) and for the group of its controls (solid lines).

cycle. The diurnal maximum for the experimental group occurred one hour earlier than that for the controls, and the diurnal minimum lasted for two hours (3 to 5 A.M.) in this former group as opposed to one hour (4 to 5 A.M.) in the latter, similarly indicating a small shift to an earlier time.

A comparison of the 24-hour cycles of the two December groups with those of the four groups that were studied earlier in the year (Fig. 3) shows that there are certain likenesses in form, although the temporal aspects of the curves differ. The most obvious difference is that the diurnal maximum for the December groups occurred just before and just after noon whereas the other curves had maxima later in the afternoon. The morning minimum for the December groups was also earlier, i.e., between 3 and 5 A.M., as were the minor peaks of the morning which occurred immediately after midnight. The period between the minor and major peaks, however, was again 12 hours as in groups 1, 2, and 3.

The tidal cycles of the December groups (Fig. 7) are also similar to those of

the earlier groups (Fig. 4). The two minima are separated by about 12 hours and one of the prolonged periods of increased activity is characterized by one or two hours of relatively low activity between periods of higher activity.

It is clearly apparent that the tidal cycle for the group of clams that was exposed to five periods of reversed illumination was shifted in time relative to that for the control group (Fig. 7). Each of the two minima of the experimental curve occurred about five hours later than the comparable lows of the control group. Again, in this figure, the lower average activity of the experimental animals is evident.

DISCUSSION

The evidence at hand supports the assumption that the long-cycle rhythms illustrated in Figures 1, 2, and 5 are actually lunar cycles or parts thereof. The curve drawn between points contributed by groups 1 and 2 shows almost exact lunar periodicity; the maxima are 29 days apart and the minor peak occurs halfway between the two major ones. The time to minimal activity following the minor peak of February 21 and preceding the minor peak of March 7 (Fig. 1) appears shorter than the comparable times for groups 3 and 4 (Fig. 2). Since, however, the overall activity for the clams of group 2 was greater than that for those in group 1, it is suggested that the point for February 25 really constitutes that of minimal activity. On this basis, the time for passage from the lesser maximum to the minimum would be three to four days in every instance.

It was first thought that the clams of group 3 might have been collected from the same region of Chesapeake Bay as those of groups 1 and 2. If these three groups of animals were in tidal, and consequently in lunar synchrony a major peak of activity would be expected to have occurred about April 5 and a minor one about April 20. It is seen from inspection of Figure 2A that this is not the case, but rather an apparent decline from high activity characterizes the cycle about April 5 and a minor peak appears on April 14.

Several explanations for this diversity are plausible. It is very probable that the animals of group 3 had been collected from an area of Chesapeake Bay in which the times of tides differ from those in the region from which the clams of groups 1 and 2 had been obtained. The times of tides in different parts of Chesapeake Bay vary quite considerably because of the topography of this area. Secondly, it is possible that all these animals were collected in the same region but that the rhythms for group 3 were put out of phase by light, temperature, or some other stimulus to which the clams were subjected during shipment from the Atlantic coast to Evanston.

No correlations between the phases of the tidal cycle for group 3 and actual tidal events could be made. Therefore, it is impossible to know whether or not the phases of this tidal rhythm were shifted. Also, the diurnal cycle of activity for group 3 did not differ basically from those for groups 1 and 2. Unpublished observations suggest that for fiddler crabs, the persistent diurnal and tidal rhythms, the summation of which determines the phases of the long-cycle rhythms, are shifted together by temperatures that approach freezing. This is also true of the cycles for fiddler crabs that are shifted by exposure to an illumination of high intensity (Brown, Fingerman, Sandeen and Webb, 1953). Nevertheless, the data on the effects of changes in illumination on the rhythms for quahogs, reported in the present

paper, appear to indicate that the diurnal and tidal cycles were affected differentially by this treatment.

It was seen that the period of minimal activity for group 4 was correlated roughly with the semi-lunar low, low tides which occurred just after the full moon of June 26. It was therefore thought that the long-cycle rhythms for the other groups also might be correlated directly with phases of the moon. This does not seem to be the case, however, since a full moon occurred on February 28, 1953, or about seven days after a minor peak for group 1, on March 28, 1953, or about two weeks before a minor peak for group 3, on June 26, or two days after a minor peak for group 4, and on December 20, or about four days after a minor peak for the group of clams that served as controls in the experiment on effects of reversed illumination. These facts suggest that it is the time of the local tides which determines the phase relationships of the semi-lunar and lunar cycles rather than a more direct lunar influence. This hypothesis is supported by results of the study of the tidal rhythms of color change for *Uca* (Brown, Fingerman, Sandeen and Webb, 1953), which demonstrated that the cycles of crabs that inhabit areas, the tidal times of which differed by about four hours although located in the same geographical region, were correspondingly four hours out of phase with one another. Therefore, the phases of the long-term cycles for quahogs that live in areas of Chesapeake Bay which differ in times of tides would likewise differ from one another, although all the animals would be exposed to the same phases of the moon at the same times.

The similarity of the curve for the tidal cycle for group 4 to those for groups 1, 2, 3, and the group that served as controls in the illumination experiment, and the correlation of the phases of the rhythm of group 4 with actual tidal events, suggest strongly that this same correlation could be made for the latter groups if the exact data on tides were available. All the groups showed diurnal cycles of activity. The investigation by Brown, Fingerman, Sandeen and Webb (1953) proved conclusively that the presence of both persistent tidal and diurnal rhythms in the same animal produces a persistent semi-lunar rhythm since the tidal cycle is in the same relationship with the diurnal cycle at 14.8-day intervals.

If the effect of alternate tidal cycles on metabolism or activity differs quantitatively or qualitatively from that of the intervening tidal cycles, the tidal cycles would be in a similar relationship with the diurnal cycle only at 29.5-day intervals, or lunar ones. This situation appears to be true for the long-term rhythm of the quahogs, since the alternate semi-lunar maxima are larger than the intervening ones. The activity data for only 15 consecutive days were used in the analysis of each of the tidal rhythms. Therefore, when the data for the first 14 days were repeated in the analysis, values which included the effects of both of the daily tides were averaged, and any difference between them was obscured. The tidal rhythm of O_2-consumption for the snail, *Littorina littorea* (Sandeen *et al.*, 1954), was analyzed in the same manner as those reported in the present paper, and it appears to show more clearly than those for the groups of quahogs that the effects of the alternate tidal cycles are not equivalent.

At present, the diurnal cycle of activity of the quahogs cannot be characterized precisely. As has been seen, the activity generally reached a maximum during the afternoon hours and minimal activity occurred during the morning hours. It is possible that the differences that were seen among the diurnal rhythms for the various groups reflect adaptations of the diurnal activity to the varying lengths of

daylight throughout the year. The cycles for groups that were observed during March, April, June, and December show indications that during periods of shorter day-length the greater activity of the clams occurred earlier in the day than it did during the times of the year when days are longer. Activities for groups 2 and 3 which were observed in March and April declined after 4 P.M. Group 4, which was studied in June, showed an hour of maximal activity between 5 and 6 P.M. Correspondingly, the diurnal cycle for the controls for the animals that were exposed to light and dark and were observed in December reached a maximum just after 12 noon. The activity decreased rather rapidly after this time. If this assumption is correct, it is seen that the rhythm for group 1 is not in line with those of the other groups. Group 1 was observed in February at which time daylight hours are fewer than in March and April in the northern hemisphere, but its period of maximal activity extended from 5 P.M. until 9 P.M.

The periods of minimal activity for the different groups of animals also show some correlation with the day-length that existed at the times of observation. For groups 1, 2, and 3, which were observed during the early spring, the diurnal lows were recorded between 9 and 10 A.M., at which times the groups were active 0%, 22%, and 13% of the hour. The period of low activity for group 4, studied in June, ended shortly after 6 A.M., and at this time these animals were active 25% of each hour period. The cycle for the December control group does not fit into this series. Here the morning minimum was reached between 4 and 5 A.M. when the clams were open 16% of the hour. However, these varying diurnal rhythms may be indications of an annual cycle, and yet not reflect day-length directly.

The data for the persistent diurnal rhythms of O_2-consumption for marine snails (Sandeen *et al.*, 1954) and for fiddler crabs (Brown, Bennett and Webb, 1954) suggest that the diurnal patterns may change slightly in accordance with day-length. In these cases, as in the present report, the animals were maintained under constant laboratory conditions. Brown and Stephens (1951) have shown that the day-phase of the diurnal cycle of color change for *Uca* can be influenced by the length of the daily photoperiod to which series of crabs are exposed. The changes in this cycle, once induced, persisted after the animals were placed in constant darkness.

It is interesting to note, once more, that an earlier study of *Venus mercenaria* in its natural environment did not yield any positive suggestions of rhythmic activity (Loosanoff, 1939). It is possible that in an organism's natural habitat, characteristics of the basic persistent rhythms, *e.g.*, frequency, form, and amplitude, are masked or overshadowed by varying external stimuli such as light, temperature, and movements of the water. A suggestion that this may be true is found in the tremendous increase in the amplitude of the diurnal rhythm of color change in *Uca pugnax* after the crabs have been brought from the field and maintained in constant darkness (see Figure 10, Brown, Fingerman, Sandeen and Webb, 1953).

As is true for the persistent rhythms for fiddler crabs, those for *Venus* can be modified to some degree by exposing the animals to alternating periods of darkness and illumination. It has been shown that five consecutive days (8 A.M. to 8 P.M.) of darkness with five nights (8 P.M. to 8 A.M.) of illumination of 100 ft. c. effected changes in the diurnal, tidal, and long-cycle rhythms of activity for a group of clams. Whether or not the apparent shift of the diurnal cycle is significant is difficult to state. As discussed previously, the diurnal rhythms for the different groups of animals that were observed varied to the extent that a precise characterization of this

rhythm could not be made. Furthermore, it is not known whether these variations reflected adaptations to day-length. Therefore, the diurnal cycles of activity illustrated for the experimental group and its control (Fig. 6) may be in phase with one another. However, the moving of both the morning minimum and the afternoon maximum for the experimental group by about the same amount of time and in the same direction, *i.e.,* one hour earlier than the comparable points for the control group, seems to indicate that the phases of this cycle were shifted.

The effects of five consecutive days of darkness and five nights of illumination on the tidal and long-term cycles of activity can be discerned more readily. The tidal rhythm for the experimental group seems to be shifted so that the low points occurred five hours later than the comparable points for the control group (Fig. 7). In other words, the tidal cycle of the experimental animals is about five days ahead of the control one. The phases of the long-term cycle for the experimental clams occurred four days after those of the controls (Fig. 5). Since the long-cycle rhythm is the result of the moving of the tidal influence across the diurnal cycle at the rate of about 50 minutes per day, or so that the tidal effect occurs 50 minutes later on each successively later day, the apparent five-hour shift of the tidal rhythm could not have effected a shift of the long-cycle rhythm such that its phases occurred four days late. If the experimental tidal rhythm had been shifted so that on a hypothetical day the low point of the cycle, which probably correlates with the time of low tide in the natural habitat of the clams, occurred between 11 P.M. and midnight and the comparable low point for the control group occurred five hours earlier, or between 6 and 7 P.M. on the same day, the resulting long-term cycle for the experimental animals would be moved so that its phases would occur earlier rather than later than those of the cycle for the control group.

In order that the phases of the long-cycle rhythm for the experimental group be four days late when compared to those of the control group, the tidal cycle for the former would have had to be shifted so that a low point occurred about four to five hours earlier than, or that the tidal cycle of the experimental group was four to five days behind, that of the control group. As has been seen, this was not the case (Fig. 7). However, when a particular point of the experimental cycle which is the result of a tidal influence appears to be five hours late, or about four to five days ahead, it may in reality be nearly 20 hours early, since a particular tide recurs about every 24.8 hours. If this situation exists, the phases of the long-cycle rhythm for the experimental animals would be expected to occur about 25 days before those of the control cycle (Fig. 8). The facts that a particular tide occurs about 50 minutes later on each successively later day, and that the long-cycle rhythm is repeated about every 29 days, account for this relationship.

Several hypotheses may be offered to explain these shifts. 1) The first dark-to-light or the first light-to-dark change may have been the stimulus that effected the movement of the phases of the tidal cycle for the experimental group about 20 hours backwards, and consequently, the phases of the long-cycle rhythm 25 days ahead of those for the control clams. 2) Possibly, the effects of the changes of illumination or the periods of high illumination or darkness were cumulative, and thereby caused the shifts that have been discussed. On the basis of the results of previous investigations it is probable that the changes from darkness to light and the light period were the effective factors. Webb (1950) found that the phases of the diurnal rhythm of color change for fiddler crabs that were maintained in con-

stant darkness were shifted by single illumination periods of varying lengths.   The
direction and the amount of shift were dependent on the time of day at which the
animals were exposed to an illumination of 90 ft. c.   A more recent paper (Brown,
Fingerman and Hines, 1954) presents an hypothesis to account for the graded series
of shifts of the diurnal cycle of color change for *Uca*.   These graded shifts in the
phases of the cycle were noted when series of crabs were exposed to various higher
illuminations by night and various lower ones by day.   Two factors appeared to
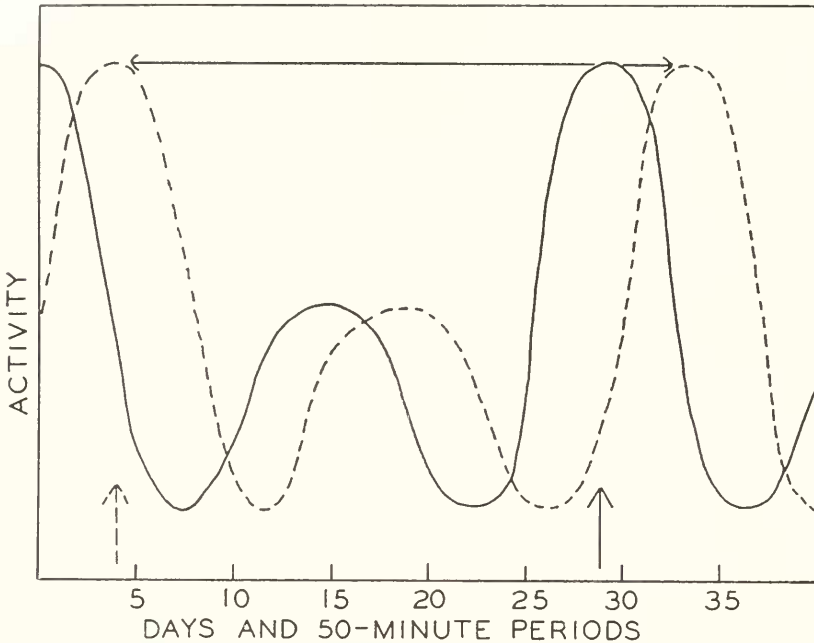operate in the altering of the phases: 1) the strength of the light-increase stimulus,

FIGURE 8. An illustration to show the effects of shifts of the tidal cycle of an experi-
mental group of clams on the long-term rhythm of activity. The broken line curve is that of
the experimentals and the solid line one is that of the controls. The broken line arrow indi-
cates the time of low tide for the experimental group and the solid line one for the control
group. The horizontal arrows indicate the directions and amounts of shifts possible. (For
discussion, see text.)

and 2) the brightness of the higher illumination to which the animals were exposed
by night.
    Exposures of fiddler crabs to several cycles of altered illumination operate to
shift the tidal rhythms of color change as well as the diurnal cycles (Brown, Finger-
man, Sandeen and Webb, 1953).   Here the phases of the tidal and the diurnal
rhythms were moved in the same direction and by the same number of hours.   In
this case, the temporal relationship which existed between the two cycles at the
times of exposure of the crabs was maintained.   In the present study, the data lead
to the assumption that the tidal rhythm for the experimental group of quahogs was
shifted so that its phases occurred about 20 hours earlier than those for the animals

that were exposed to constant low illumination. The shift of the diurnal rhythm was, at most, only one hour in the same direction. The diurnal cycle could have been shifted by a number of days, however, and this could not have been ascertained. For example, if each of the five alternating light cycles had shifted the diurnal rhythm backwards by five hours, it would appear to have been set backwards only one hour. Meanwhile, if the tidal influence had each day moved forward 50 minutes over the diurnal cycle while each day the diurnal cycle was set backwards five hours, the phases of the tidal cycle would be in effect set backwards about 20 hours during the five days. This interpretation is so clearly compatible with the second hypothesis advanced earlier that its likelihood is great.

From the results of the present and earlier investigations, it is evident that light stimuli are effective in setting phases of both persistent diurnal and tidal rhythms, and consequently the semi-lunar and lunar ones. Two other investigators recently have discussed the problem of setting the phases of persistent cycles. Brett (1954) saw that a light flash of only one minute was sufficient to set the phases of the diurnal rhythm of emergence for *Drosophila*. The tidal movements of the water seem to participate in determining the phases of the tidal rhythm for *Mytilus* (Rao, 1954).

The question remains: are these persistent rhythms endogenous or exogenous? If they are endogenous, or dependent upon basic physiological mechanisms, the various experimental procedures which shift the phases of the cycles have actually altered the "internal clocks." On the other hand, if the rhythms are controlled by exogenous forces, the manifestations of the rhythms have merely been moved from their normal relationships with the basic cycles when shifts are apparent. The present study contributes no evidence concerning this problem, but studies planned for the near future should provide an answer.

Precise rhythms of activity such as those discussed in the present paper are seemingly adaptive for this species of clam. Loosanoff (1937) states that the gonads of adult *Venus mercenaria* inhabiting Long Island Sound appear to contain ripe sperm or ova throughout the greater part of the year. Yet, spawning does not occur in the majority of animals until August. A certain critical water temperature has been thought to be the stimulus for spawning (Loosanoff, 1937). Although temperature may be a limiting factor it seems more probable that the presence of persistent lunar rhythms, resulting from persisting diurnal and tidal ones in these clams, may eventually be shown to be the immediate regulator of spawning activities. Such a mechanism would provide more precision than could be obtained by the very slow, gradual temperature change of the water.

The greater activity of the clams during the day than during the night may be adaptively correlated with the availability of food. Plankton populations, which supply much of the food of this species, are known to undergo diurnal rhythms of migration. In general, the greatest density of plankters near the surface is found during the hours of the night, and conversely, the greatest density at greater depths occurs during daylight (Russell, 1927).

Summary

1. Continuous kymograph recordings of the opening and closing of the valves of common quahogs, *Venus mercenaria*, secured from the Virginia coast and from

Woods Hole, Massachusetts were made during February, March, April, June, and December, 1953.

2. The activity records of these clams maintained under constant laboratory conditions show a persisting diurnal rhythm with maxima and minima occurring in the afternoon and early morning, respectively. There is some evidence that the pattern of the diurnal cycle varies with the time of year.

3. A persisting tidal rhythm is also evident. In the group of animals for which correlations with actual tidal phases could be made, it was seen that the times of minimal activity in this cycle correspond closely with the times of low tide in the area from which the animals were collected.

4. The summation of these two cycles, diurnal and tidal, is responsible for an observed lunar cycle. The phases of the lunar cycle are probably set directly by the tides to which the animals are exposed rather than by any direct lunar influence.

5. The rhythms of a group of clams that was exposed to darkness during the day (8 A.M. to 8 P.M.) and to an illumination of 100 ft. c. during the night (8 P.M. to 8 A.M.) for five consecutive 24-hour periods show shifts in their cycles when compared to those of control animals. The shifts are such that the phases of the diurnal rhythm occurred one hour earlier than those of the controls; the phases of the tidal cycle occurred about 20 hours earlier than those of the controls; and the phases of the long-cycle rhythm were, in consequence, 25 days ahead of those of the controls.

6. The manner in which these shifts of the phases of the cycles are related is discussed and illustrated.

7. Adaptive significances of these persistent rhythms in these clams are suggested.

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